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Assignment tests, telemetry and tag-recapture data converge to identify natal origins of leatherback turtles foraging in Atlantic Canadian waters

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Summary

1. Investigating migratory connectivity between breeding and foraging areas is critical to effective management and conservation of highly mobile marine taxa, particularly threatened, endangered, or economically important species that cross through regional, national and international boundaries.

2. The leatherback turtle (*Dermochelys coriacea*, Vandelli 1761) is one such transboundary species that spends time at breeding areas at low latitudes in the northwest Atlantic during spring and summer. From there, they migrate widely throughout the North Atlantic, but many show fidelity to one region off eastern Canada, where critical foraging habitat has been proposed. Our goal was to identify nesting beach origins for turtles foraging here.

3. Using genetics, we identified natal beaches for 288 turtles that were live-captured off the coast of Nova Scotia, Canada. Turtles were sampled (skin or blood) and genotyped using 17 polymorphic microsatellite markers. Results from three assignment testing programs (ONCOR, GeneClass2 and Structure) were compared. Our nesting population reference data set included 1417 individuals from nine Atlantic nesting assemblages. A supplementary data set for 83 foraging turtles traced to nesting beaches using flipper tags and/or PIT tags (n = 72), or inferred from satellite telemetry (n = 11), enabled ground-truthing of the assignments.

4. We first assigned turtles using only genetic information and then used the supplementary recapture information to verify assignments. ONCOR performed best, assigning 64 of the 83 recaptured turtles to natal beaches (77·1%). Turtles assigned to Trinidad (164), French Guiana (72), Costa Rica (44), St. Croix (7), and Florida (1) reflect the relative size of those nesting populations, although none of the turtles were assigned to four other potential source nesting assemblages.

5. Our results demonstrate the utility of genetic approaches for determining source populations of foraging marine animals and include the first identification of natal rookeries of male leatherbacks, identified through satellite telemetry and verified with genetics. This work highlights the importance of long-term monitoring and tagging programmes in nesting and high-use foraging areas. Moreover, it provides a scientific basis for evaluating stock-specific effects of fisheries on migratory marine species, thus identifying where coordinated international recovery efforts may be most effective.

Key-words: combine methods, homing, international, migrations, stock structure, validation

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Introduction

Many marine species undertake long-range seasonal migrations between breeding grounds and foraging areas that involve crossing regional, national and international boundaries. Furthermore, animals may occupy diverse habitats during different life-history stages. Therefore, linking the distribution of animals across developmental or feeding areas to breeding areas, which may be thousands of kilometres apart, is fundamental to accurately defining the geographical boundaries of the management units of interest. The transboundary migrations undertaken by marine taxa require international cooperation for management and conservation (Dutton & Squires 2008, 2011; Patino-Martinez *et al.* 2008; Jodice & Suryan 2010). Where one country may be responsible for managing animals in breeding areas, another country may need to manage threats in foraging habitats. Understanding and conserving this migratory connectivity (Webster *et al.* 2002) requires participation and buy-in from many parties and constituents through multi-agency and multi-jurisdictional cooperation and communication (Jodice & Suryan 2010).

Identifying source populations of foraging animals is important because individuals from different populations may mix on foraging grounds. Only by understanding both the magnitude of threats to a species on its foraging grounds, and the natal origins of individuals represented in a foraging population, can we begin to link threats (or efforts to mitigate threats) to trends in source populations. For both terrestrial and marine taxa, threats on foraging grounds have been linked to precipitous declines in breeding populations. For example, some pelagic sea birds are vulnerable to incidental capture in longline fisheries in foraging areas distant from their breeding colonies. Although estimates of fishing-related mortality have been associated with declines in some colonies, for example albatrosses (Baker *et al.* 2007), for others, quantification of specific threats have not been linked to observed declines in source populations; the declines remain largely unexplained (Reid *et al.* 2004).

Traditional tagging and satellite telemetry have been used to investigate movements of marine animals across political boundaries and between habitats, and associated studies have vastly improved our knowledge of life history and migration strategies for many species (Lutcavage *et al.* 2000; Thompson, Moss & Lovell 2003). Satellite telemetry in particular has been essential in advancing knowledge of the movements of wide-ranging threatened or endangered species, such as apex predators in the Pacific (Block *et al.* 2011). In addition, genetic techniques have greatly improved our understanding of population structure and the mobility of marine taxa. Both maternally inherited mitochondrial (mt) DNA and nuclear DNA (microsatellites or SNPs) have been used to define stocks of sperm whales (*Physeter macrocephalus*) (Engelhaupt *et al.* 2009; Mesnick *et al.* 2011), investigate colonization events by Canada geese (*Branta canadensis*) (Scribner *et al.* 2003), and to assess the level of gene flow between polar bear (*Ursus maritimus*) populations (Paetkau, Amstrup & Born 1999). Using genetic stock identification (GSI), mixed-stock analysis and single nucleotide polymorphisms (SNPs), managers have the ability to evaluate the strength of sockeye salmon stocks in Bristol Bay, Alaska, just a few days before the fish migrate to river mouths, where the fishery occurs. Fishery openings or closures may be adjusted in nearly real-time (within 2–4 days depending on weather) according to which stock is most abundant at the time of sampling (Dann *et al.* 2009; Sagarin *et al.* 2009). This practice ensures that no single stock is targeted disproportionately and that the fishery is managed optimally. For highly migratory species and for fish species in particular, the conventional way of determining stock composition on foraging grounds has been mixed-stock analysis (MSA) using mtDNA. Although MSA has been a useful tool for managing fisheries, the application of MSA to rare or highly migratory marine species has proven more difficult because of the sample size needed to get good quality results with small confidence limits and good statistical power. Recently, Bowen *et al.* (2007) used MSA to investigate hawksbill turtle (*Eretmochelys imbricata*) migrations and contributions of source nesting beaches to foraging areas in the Caribbean, while Okuyama and Bolker (2005) used a Bayesian hierarchical model with ecological covariates to refine estimates for mixed stocks of green (*Chelonia mydas*) and loggerhead turtles (*Caretta caretta*). Although MSA identifies the proportions of a foraging population that belong to source populations, assignment testing (AT) programmes provide a means of assigning targeted individuals to source populations (Manel, Gaggiotti & Waples 2005). For example, source populations were identified for two albatross species (*Thalassarche cauta* and *T. steadi*) caught and killed incidentally in fisheries off New Zealand, South Africa and Australia using assignment tests (Abbott *et al.* 2006), and Hanson *et al.* (2010) traced resident killer whale (*Orcinus orca*) prey (mainly *Oncorhynchus tshawytscha*) to spawning regions of origin.

In combination, tagging and genetics provide a powerful way to identify linkages between habitats used by marine species. This approach has recently been used for examining green turtle migratory connectivity (Godley *et al.* 2010). For other marine turtles, the linkages are not quite as clear. Leatherback turtles (*Dermochelys coriacea*) of the western North Atlantic nest at low latitudes and exhibit natal homing (Dutton *et al.* 1999, 2005). They make long-distance migrations to breeding and nesting grounds yearly for males (James, Eckert & Myers 2005) and every 2–3 years or more for females (Stewart & Johnson 2006), and then forage on gelatinous prey (Heaslip *et al.* 2012) throughout the North Atlantic (James, Ottensmeyer & Myers 2005; Eckert *et al.* 2006;

Hays *et al*. 2006; Fossette *et al*. 2010a,b). One of the foraging regions for northwest Atlantic leatherbacks is off eastern Canada (Fig. 1), where critical foraging habitat has been proposed. The ability to maintain body temperatures well above ambient (James & Mrosovsky 2004; Bostrom & Jones 2007) allows leatherbacks to exploit these northern niches successfully. Although satellite tracking studies have followed leatherbacks from Canadian foraging grounds (James, Ottensmeyer & Myers 2005) to locations throughout the Atlantic, and tag-recapture data have enabled identification of some source populations (James, Sherrill-Mix & Myers 2007), the natal origins of these turtles are generally not known, as is the relative contribution of different nesting colonies to the Canadian foraging population. Recently, Dutton *et al*. (in press) found population structuring for nine nesting populations of leatherbacks in the Atlantic based on nuclear data (microsatellites) and concluded that these nesting assemblages represent Demographically Independent Populations (DIPs).

Intensive conservation work focused on several key Atlantic leatherback nesting areas has helped address local threats, including egg poaching, predation and habitat loss (Chacón-Chaverri & Eckert 2007; Thomé *et al*. 2007; Fossette *et al*. 2008). However, the efficacy of nesting beach conservation work is limited by the species' biology and distribution, as only mature females and their eggs can benefit directly and the vast majority of the species' life history is spent elsewhere. Advocacy for leatherback threat assessment in critical marine habitat by nesting beach programmes and the countries that host nesting populations has traditionally been precluded by insufficient knowledge regarding the foraging destinations

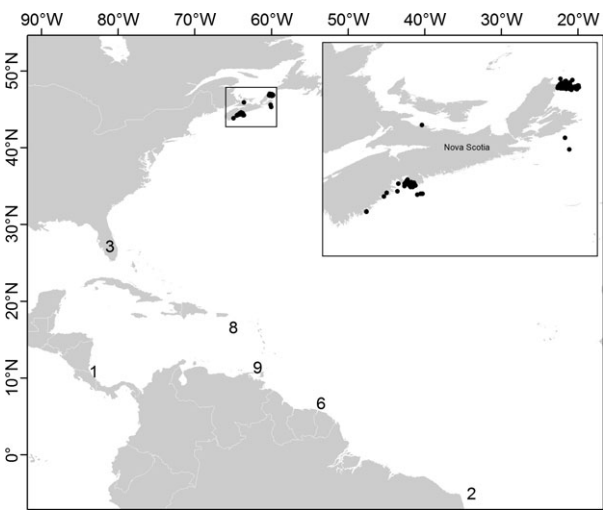

Fig. 1. The location of leatherback nesting populations throughout the Western Atlantic and foraging habitat (and the study area) in Canadian waters (inset). 1 = Atlantic Costa Rica, 2 = Brazil, 3 = Florida, 4 & 5 = Gabon & Ghana (not shown) 6 = French Guiana, 7 = South Africa (not shown), 8 = St. Croix, USVI, and 9 = Trinidad. The black dots represent leatherback turtle capture locations off Nova Scotia.

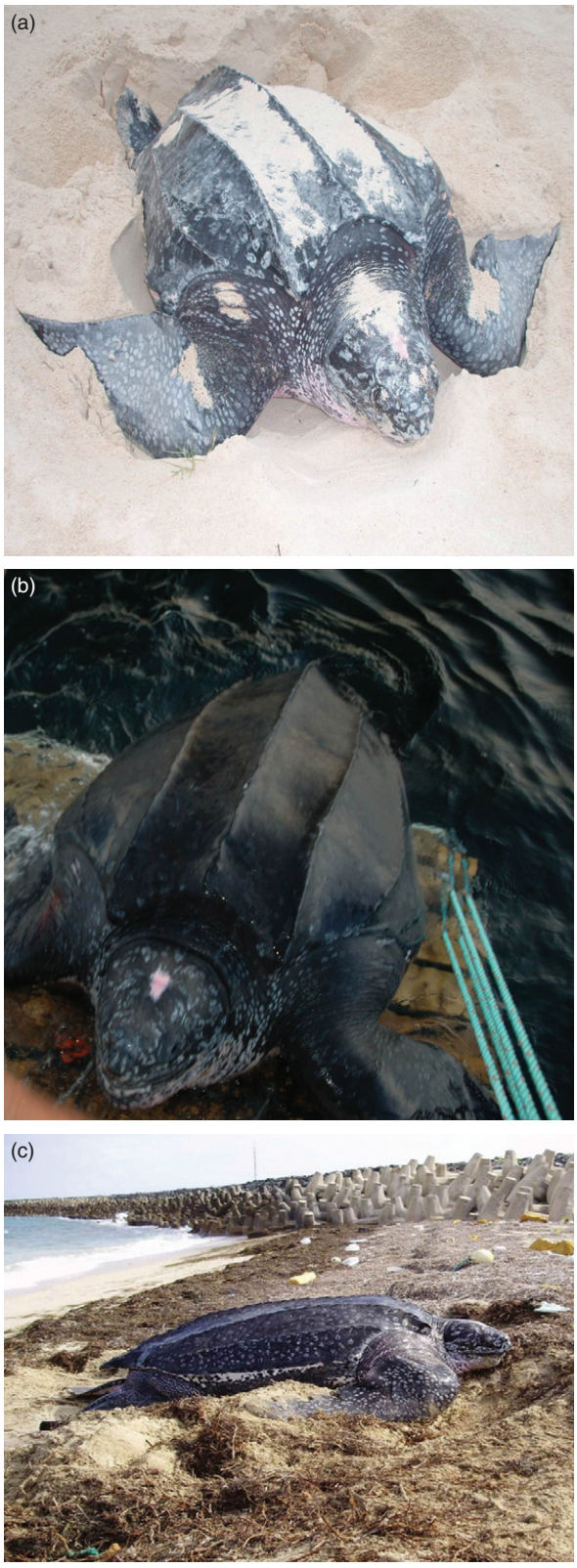

Fig. 2. Leatherback nesting origins confirmed through tag recapture (in this case, flipper tags). Female leatherback nesting (and tagged) 26 April 2007 in Anguilla, BVI (a), then recaptured off Nova Scotia, Canada, 6 September 2007 (b), and recaptured a third time in 2009 at Hovensa, St. Croix, USVI (c). Photos courtesy of James Gumbs (a), Canadian Sea Turtle Network (b) and Claudia Lombard, USFWS (c).

of leatherbacks from specific rookeries. Although other foraging areas may not be as well studied (e.g. Fossette *et al*. 2010a), Canadian waters do host one of the largest seasonal foraging aggregations of leatherbacks in the North Atlantic (James *et al*. 2006), and it is critical to understand the demographic linkages with nesting populations to evaluate how threats in this region may impact different nesting populations throughout the Atlantic. In addition, this high-latitude foraging ground supports adult turtles as well as sub-adults (James, Sherrill-Mix & Myers 2007). Identifying the natal origin for these younger age class turtles has not been not possible using traditional tags or even satellite telemetry. Genetic techniques thus present a rare opportunity to determine the source for immature turtles, as well as for the adults.

Using nesting assemblage baseline genetic data for nine populations (Dutton *et al*. in press), microsatellite nuclear DNA markers and resightings data (flipper tags and satellite telemetry), the purpose of this study was to identify natal origins for 288 leatherback turtles (males, females and juveniles) captured from 2001 to 2012 off Nova Scotia, Canada.

Materials and methods

FIELD SAMPLING

Tissue biopsies were obtained from live-captured and stranded leatherback turtles in Nova Scotia, Canada (Fig. 1). Leatherbacks were live-captured using a breakaway hoop-net deployed from ~11 m commercial fishing boats equipped with a bowsprit (James, Ottensmeyer & Myers 2005) (Fig. 1). Sampling occurred from July to September, 2001–2012. For all turtles, curved carapace length (CCL), curved carapace width (CCW) and three metrics of tail length were recorded. For turtles with CCL > 145 cm, which is smaller than the average size for females nesting in the Atlantic, but still well within the size range for adult leatherbacks (Stewart, Johnson & Godfrey 2007), sex was assigned based on the tail length. Total tail length of mature male leatherbacks is normally at least twice that of females of the same carapace length. Turtles < 145 cm CCL were considered subadult. Skin samples were taken from one of the front flippers using a 6-mm sterile biopsy punch (Acuderm, Fort Lauderdale, FL, USA), and when possible, a blood sample was drawn from the dorsal cervical sinus of live turtles using a needle (9 cm, 18 gauge) and a glass Vacutainer® tube containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA) (Dutton 1995). Skin samples were stored individually in cryovials in a saturated salt (NaCl) solution; blood samples were also stored in cryovials at −20 °C. Captured turtles not presenting with flipper tags and/or implanted microchips (PIT tags) were equipped with both tag types before release.

DNA ANALYSES

Standard manufacturer protocols were used for total genomic DNA extraction using one of the following methods: X-tractor Gene robot or modified DNEasy® Qiagen extraction kit (Qiagen, Valencia, CA, USA), phenol and chloroform or chloroform only (modified from Sambrook, Fritsch & Maniatis 1989), or sodium chloride extraction (modified from Miller, Dykes & Polesky 1988). We amplified DNA using Polymerase Chain Reaction (PCR) (Innis *et al*. 1990) in a 25 μL reaction volume in thermal cyclers (ABI 2720 or Bio-Rad PTC 100). Seventeen polymorphic microsatellites were used for genotyping. Details of the primer reaction schemes were as follows: LB99, 14-5, LB110, LB128, LB141, LB142, LB145, LB143, LB133, LB123, LB125, LB157, LB158 (Roden & Dutton 2011), D1 and C102 (Dutton & Frey 2009) and N32 (Dutton 1995). An additional primer (D107; Dutton, unpublished) was used with the following reaction scheme: initial denaturation for 5 min at 94 °C, 35 cycles of 40 s at 94 °C (denature), 40 s at 58 °C (annealing) and 40 s at 72 °C (extension) with a final extension (5 min) at 72 °C. PCR products were assessed for amplification using ethidium bromide stain in 2% agarose gels (Maniatis, Fritsch & Sambrook 1982) and analysed using an ABI Genetic Analyzer (Prism 3730 or 3100) using ROX500 fluorescent size standard (PE Applied Biosystems, Foster City, CA, USA). GeneMapper 4·0 (Applied Biosystems, Foster City, CA, USA) was used for scoring alleles, with each allele call being manually verified. Following recommendations (Bonin *et al*. 2004) to ensure high-quality data, we ran positive and negative controls with each DNA extraction plate. All PCRs contained negative controls as well, and products were analysed alongside samples. Micro-Checker version 2.3.3 (Van Oosterhout *et al*. 2004) was used to assess allelic stutter, large allele dropout and null alleles in the foraging turtle data set.

ASSIGNMENT TESTING

We used microsatellite allele frequencies from nine genotyped baseline populations as the reference data set for assignments. This data set comprises the currently known stock structure for nesting leatherbacks in the North Atlantic (Dutton *et al*. in press). The nine populations represent both western and eastern Atlantic nesting sites and include: Atlantic Costa Rica; Brazil; Florida; Gabon; Ghana; French Guiana (inclues Suriname); South Africa; St. Croix (USVI); and Trinidad. Individuals (n = 1417) included in the reference data set (Table 1) had genotypes for at least 12 of 17 microsatellite markers, and all were female. The reference data set was evaluated for null alleles, linkage disequilibrium (Roden & Dutton 2011), and for Hardy-Weinberg equilibrium (Dutton *et al*. in press). For the H-W analysis across all populations, none of the 17 loci deviated from equilibrium ($P < 0{\cdot}05$) (Dutton *et al*. in press. Population

Table 1. Nesting female leatherback sample sizes for each of the nine Atlantic populations, that were used as the reference data set. Summary statistics (including F_{ST} and F'_{ST}) for the reference data set may be found in Dutton *et al*. (in press)

Source Rookery	*n*
1. Atlantic Costa Rica	323
2. Brazil	21
3. Florida	160
4. Gabon	207
5. Ghana	51
6. French Guiana	167
7. South Africa	39
8. St. Croix (USVI)	368
9. Trinidad	81
Total	1417

differentiation was calculated several different ways (χ^2, F_{ST} and F'_{ST} values; Dutton *et al*. in press) and based on the microsatellite analyses, nine Demographically Independent Populations (DIPs), as listed above, were defined.

Two sets of data informed our stock assignments for foraging turtles. The first comprised genotypes of the 288 turtles sampled in Nova Scotia, Canada. The second data set contained records from 72 of these turtles where the nesting beach origin was confirmed from detection of flipper tags and/or microchips (PIT tags) either at the time of capture in Canada, or subsequently on a nesting beach; or inferred from those turtles equipped with satellite-linked tags that were tracked to, and exhibited seasonal residency in waters directly adjacent to nesting areas ($n = 11$; nine males, two females). For tracked individuals, we assumed that residency (normally a minimum of 2 weeks) adjacent to a specific nesting beach (or group of proximate beaches) prior to and/or during the nesting season indicated they were targeting that nesting area. These turtles were thus considered recaptured. This combined information of tag recaptures and satellite telemetry data was used to ground-truth nesting beach assignments, and to assess which assignment programme performed best.

We used assignment testing (AT) programs ONCOR (Kalinowski, Manlove & Taper 2007), GeneClass2 (Piry *et al*. 2004), and Structure version 2.3.4 (Pritchard, Stephens & Donnelly 2000) to determine the most likely nesting stock origin of the 288 turtles sampled in Canadian waters. In ONCOR, we loaded the reference data set and then used our foraging data set (as the mixture population) along with the Individual Assignment option to assign each turtle. GeneClass2 assigned a population for each foraging turtle using an assignment threshold of 0·05, with Rannala & Mountain (1997) as our criteria for computation. We used all 17 loci for the assignments, as an earlier exploration of the data set using the Genetic Algorithm-based Feature Selection-GAFS (Topchy, Scribner & Punch 2004) revealed that the inclusion of either 16 or 17 loci would optimize accuracy of the assignments. For Structure, the assignments with the highest probabilities were achieved with a burn-in period of 10 000 iterations, followed by 10 000 Markov chain Monte Carlo replicates. We used prior population information to assist with clustering (from the reference data set sampled on specific nesting beaches). For individuals with no prior population information (foraging turtles), we assumed a no-admixture model. We followed the original model in Pritchard, Stephens & Donnelly (2000), assuming that the allele frequencies were independent with a lambda value of 1·0 to guide the assignment of the foraging turtles.

We used the second data set (tag resightings and tracks of satellite-tagged turtles) to evaluate the three AT programmes. The number of correct assignments to known nesting beach origin was used as a basis to rate the performance of each programme.

Results

FIELD SAMPLING AND DNA ANALYSES

A total of 288 turtles were sampled in Canada over 12 years (272 live-captured and 16 stranded). Of these, 177 were females, 83 were males, and 28 were sub-adult (unknown sex) (Table 2). The average size (± SD) was 150·7 ± 9·1 cm CCL and 109·8 ± 8·6 cm CCW (Table 2). Assessing microsatellite loci quality with Micro-Checker version 2.2.3 revealed that although there was no evidence of large allele dropout or allelic stutter, there were null alleles detected at LB99, LB128, LB133 and LB157. The presence of null alleles in the foraging turtle data set is not a substantial concern, because although turtles might be assigned with slightly less power (Carlsson 2008), the overall results of the assignments would not change considerably (e.g. 2·4% in GeneClass2, Carlsson 2008). Summary statistics (# alleles, expected and observed heterozygosity and Hardy-Weinberg equilibrium *P*-values) for the 17 microsatellite loci from the Nova Scotia turtles are found in Table 3.

ASSIGNMENT TESTING

Using ONCOR, the majority (164) of the 288 foraging turtles were assigned to Trinidad, while 72 were assigned to French Guiana (Table 4). Costa Rica was assigned 44 turtles, 7 turtles were assigned to St. Croix and 1 turtle was assigned to Florida (Table 4). No turtles were assigned to the eastern Atlantic rookeries (Gabon, Ghana, or South Africa) or to Brazil. The results from GeneClass2 and Structure are reported in the supplementary material because we found that ONCOR correctly assigned more of the recaptured turtles than the other two programmes (see below). For GeneClass2, 109 turtles were assigned to Trinidad and 100 turtles were assigned to the French Guiana nesting aggregation. Thirty-nine turtles were assigned to Costa Rica, 18 turtles to Florida and 22 turtles to St. Croix (Table S1, see Supporting Information). The results from Structure (Table S2), showed that French Guiana had the highest number of turtles assigned (127), followed by Trinidad (69), Costa Rica (52), St. Croix (23), and Florida (17). Importantly, for both GeneClass2 and Structure, as for ONCOR, none of the 288 turtles assigned to any of the three Eastern Atlantic rookeries (Gabon, Ghana or South Africa), not

Table 2. Summary statistics for the 288 leatherback turtles captured while foraging in Canadian waters. The numbers of recaptured females, males and turtles of unknown sex (subadult) are indicated, as well as the average curved carapace length (CCL, in cm) and standard deviation, and the curved carapace width (CCW, in cm) and standard deviation. Sample size is included in parentheses

Sex	Captured	Recaptured	CCL ± SD (*n*)	CCW ± SD (*n*)
Female	177	74	152·2 ± 8·1 (161)	111·3 ± 8·6 (160)
Male	83	9	151·3 ± 8·0 (73)	110·0 ± 6·5 (72)
Subadult	28	0	135·7 ± 6·2 (20)	97·2 ± 4·5 (20)
Total	288	83	150·7 ± 9·1 (254)	109·8 ± 8·6 (252)

Table 3. Summary statistics for genotypes of the leatherback turtles captured in Nova Scotia. For each of the 17 microsatellite loci, the number of alleles, expected and observed heterozygosities, and the Hardy–Weinberg equilibrium *P*-values are given

Locus	N32	LB99	14-5	LB110	LB128	LB 141	LB142	LB 145	LB143	LB133	LB 123	LB125	LB157	LB158	C102	Dl	D107
No. alleles	5	7	9	8	3	6	5	8	5	9	5	3	14	3	3	16	2
He	0·68	0·66	0·38	0·44	0·56	0·64	0·79	0·72	0·14	0·72	0·57	0·46	0·29	0·01	0·43	0·89	0·21
Ho	0·63	0·57	0·36	0·44	0·50	0·60	0·74	0·71	0·13	0·65	0·59	0·44	0·26	0·01	0·43	0·86	0·21
HW	0·11	0·01	0·04	0·63	0·02	0·69	0·13	0·83	0·07	0·43	0·48	0·73	0·00	1·00	0·30	0·31	1·00

Table 4. ONCOR assignment test results for 288 turtles foraging off Nova Scotia, Canada. Trinidad had the greatest number of turtles assigned

Rookery	Females	Males	Unknown	All turtles
Costa Rica (> 90%)	6	0	1	7
Costa Rica/Florida	0	0	0	0
Costa Rica/St. Croix	8	3	2	13
Costa Rica/Trinidad	6	7	3	16
Costa Rica/Fr. Guiana	7	1	0	8
Total Costa Rica	27	11	6	44
Florida (> 90%)	0	0	0	0
Florida/Costa Rica	0	0	1	1
Florida/St. Croix	0	0	0	0
Florida/Trinidad	0	0	0	0
Florida/Fr. Guiana	0	0	0	0
Total Florida	0	0	1	1
St. Croix (> 90%)	2	0	0	2
St. Croix/Costa Rica	2	0	0	2
St. Croix/Florida	1	0	0	1
St. Croix/Trinidad	2	0	0	2
St. Croix/Fr. Guiana	0	0	0	0
Total St. Croix	7	0	0	7
French Guiana (> 90%)	3	4	0	7
Fr. Guiana/Costa Rica	3	1	1	5
Fr. Guiana/Florida	0	0	0	0
Fr. Guiana/St. Croix	0	0	0	0
Fr. Guiana/Trinidad	35	23	2	60
Total Fr. Guiana	41	28	3	72
Trinidad (> 90%)	13	6	5	24
Trinidad/Costa Rica	13	8	2	23
Trinidad/Florida	0	0	0	0
Trinidad/St. Croix	3	1	0	4
Trinidad/Fr. Guiana	73	29	11	113
Total Trinidad	102	44	18	164

even as a secondary designation, and none were assigned primarily to Brazil.

Using the secondary ground-truthing data set with the 83 recaptured turtles linked to nesting beaches through tag recaptures and/or satellite tracking data, we found that ONCOR accurately assigned most turtles to their known nesting beach (Table 5), with 64 correct assignments (77·1%). Of these 64, 33 were assigned to the correct assemblage, whereas 31 others could be considered correct at a regional level (Table 5, see Discussion). Nineteen turtles were assigned incorrectly, including two turtles that were confirmed nesting in Florida. In contrast, GeneClass2 and Structure correctly assigned 26 and 27 turtles, respectively, to their known nesting beach, while 34 and 31 were assigned to the correct region respectively (see Table 6). In general, assignment probabilities with GeneClass2 and Structure were lower, and the percentage of turtles correctly assigned was 72·3% and 69·9%, respectively.

Discussion

Areas of critical foraging habitat for leatherback turtles that nest in the tropics and subtropics of the western

Table 5. For 83 turtles of known nesting beach origin that were sampled in Canada, the turtle ID, sex (male, female), primary and secondary assignments with associated probabilities, and the method by which the nesting beach was identified (tags or PTT satellite tracks) are given. ONCOR assigned the natal nesting beach (or region) correctly in 77·1% of recaptured individuals

Turtle ID	Sex	Known nesting beach (females) or breeding area (males)	Ground-truth method	Primary assignment	*P* (%)	Secondary assignment	*P* (%)
Correct							
44089	F	Pacuare and Playa Negra, Costa Rica	Flipper tags	Costa Rica	95·5	Trinidad	3·3
73676	F	Captain's Bay Beach, Anguilla; Hovensa, St. Croix	Flipper tags	St. Croix	93·5	Costa Rica	6·5
78523	F	Fishing Pond, Trinidad	Flipper tags	Trinidad	93·1	Fr. Guiana	6·7
113419	F	Fishing Pond, Trinidad	Flipper tags	Trinidad	92·8	Fr. Guiana	6·7
73654	F	Grande Riviere, Trinidad	Flipper tags	Trinidad	92·3	Fr. Guiana	7·7
77396	M	French Guiana; Suriname	PTT track	Fr. Guiana	90·6	Trinidad	9·3
37409	F	Trinidad/Venezuela	PTT track	Trinidad	89·0	Fr. Guiana	10·9
77406	M	Grande Riviere, Trinidad	PTT track	Trinidad	88·1	Fr. Guiana	6·5
44085	F	Grande Riviere, Trinidad	Flipper tags	Trinidad	87·9	Fr. Guiana	11·9
37392	F	Matura, Trinidad	Flipper tags	Trinidad	87·5	Fr. Guiana	10·2
37375	F	Gandoca, Costa Rica	Flipper tags	Costa Rica	86·2	St. Croix	12·4
113421	F	Grande Riviere, Trinidad	Flipper tags	Trinidad	85·1	Fr. Guiana	14·2
37372	F	Kolokumbo and Babunsanti, Suriname	Flipper tags	Fr. Guiana	84·5	Trinidad	15·5
91175	F	Awala-Yalimapo, French Guiana; Babunsanti, Suriname	Flipper tags	Fr. Guiana	82·1	Trinidad	10·6
91169	F	Matura, Trinidad	Flipper tags	Trinidad	81·3	Fr. Guiana	17·1
37403	M	Northeast coast, Trinidad	PTT track	Trinidad	80·7	Fr. Guiana	19·3
37384	F	Matura, Trinidad	Flipper tags	Trinidad	76·7	Costa Rica	10·8
37380	F	Soropta, Panama; Cahuita, Costa Rica	Flipper tags	Costa Rica	75·9	St. Croix	23·2
44100	F	Montjoly, French Guiana	Flipper tags	Fr. Guiana	74·7	Trinidad	25·3
113418	F	Matura, Trinidad	Flipper tags	Trinidad	74·2	Fr. Guiana	24·7
113425	F	Grand Riviere, Trinidad	Flipper tags	Trinidad	72·9	Fr. Guiana	26·4
25565	M	Trinidad/St. Vincent/St. Lucia	PTT track	Trinidad	67·8	Fr. Guiana	32·2
113423	F	Fishing Pond, Trinidad	Flipper tags	Trinidad	64·8	Fr. Guiana	35·2
37379	F	Gandoca, Costa Rica; Soropta and Chiriqui, Panama	Flipper tags	Costa Rica	63·9	St. Croix	22·6
62606	F	Montjoly, French Guiana	Flipper tags	Fr. Guiana	61·3	Trinidad	38·7
112955	F	Matura, Trinidad	Flipper tags	Trinidad	61·1	Fr. Guiana	31·6
37387	F	Matura, Trinidad	Flipper tags	Trinidad	59·1	Fr. Guiana	39·9
51985	F	Grande Riviere, Trinidad	Flipper tags	Trinidad	58·3	Fr. Guiana	41·6
37397	F	Grande Riviere and Matura, Trinidad	Flipper tags	Trinidad	57·5	Fr. Guiana	40·3
91165	F	Trinidad	Flipper tags	Trinidad	57·1	Fr. Guiana	41·4
77407	F	Awala-Yalimapo, French Guiana	Flipper tags	Fr. Guiana	56·6	Trinidad	43·1
91173	F	Fishing Pond and Matura, Trinidad	Flipper tags	Trinidad	54·0	Fr. Guiana	45·9
52000	F	Grande Riviere, Trinidad	Flipper tags	Trinidad	49·7	Fr. Guiana	48·7
Regional							
112949	F	Matura, Trinidad	Flipper tags	Fr. Guiana	99·1		
112944	F	Awala-Yalimapo, French Guiana	Flipper tags	Trinidad	91·6	Fr. Guiana	8·1
91170	F	Chiriqui, Panama	Flipper tags	Costa Rica	88·8	Fr. Guiana	9·3
91168	M	Grenada/Leeward Islands	PTT track	Fr. Guiana	87·9	Trinidad	12·1
62613	F	Playa Parguito, Venezuela	Flipper tags	Trinidad	81·9	Fr. Guiana	17·8
77403	M	St. Lucia; St. Vincent; Grenada	PTT track	Fr. Guiana	79·1	Trinidad	20·4
52001	F	Levera, Grenada	Flipper tags	Trinidad	77·0	Fr. Guiana	21·9
113424	F	Cayenne, French Guiana	Flipper tags	Trinidad	75·1	Fr. Guiana	24·9
73651	F	Levera, Grenada	Flipper tags	Trinidad	73·5	Costa Rica	21·0
25552	F	Matura, Trinidad	Flipper tags	Fr. Guiana	71·7	Trinidad	28·3
44088	F	Point Isere, French Guiana	Flipper tags	Trinidad	71·6	Fr. Guiana	27·8
91163	F	Matura, Trinidad	Flipper tags	Fr. Guiana	69·0	Trinidad	29·0
44093	F	Awala-Yalimapo, French Guiana; Babunsanti, Suriname	Flipper tags	Trinidad	67·9	Fr. Guiana	31·6
73665	F	Petit Carenage Beach, Grenada	Flipper tags	Trinidad	67·5	Costa Rica	25·0
113422	F	Petit Carenage and Carriacou, Grenada	Flipper tags	Trinidad	67·0	Fr. Guiana	32·9
113416	F	Matura and Fishing Pond, Trinidad	Flipper tags	Fr. Guiana	66·8	Trinidad	27·7
44101	F	Montjoly, French Guiana	Flipper tags	Trinidad	65·4	Fr. Guiana	34·6
113430	F	Cipara, Venezuela	Flipper tags	Trinidad	65·1	Fr. Guiana	34·5
37400	M	Bocas del Toro, Panama	PTT track	Costa Rica	63·2	Trinidad	26·7

Table 5. (continued)

Turtle ID	Sex	Known nesting beach (females) or breeding area (males)	Ground-truth method	Primary assignment	*P* (%)	Secondary assignment	*P* (%)
113435	F	Cayenne: Montjoly, French Guiana	Flipper tags	Trinidad	61·4	Fr. Guiana	38·2
77405	F	Grenada/St. Vincent	PTT track	Trinidad	58·8	Fr. Guiana	31·2
77402	F	Matura, Trinidad	Flipper tags	Fr. Guiana	57·5	Costa Rica	21·2
112967	F	Fishing Pond, Trinidad	Flipper tags	Fr. Guiana	56·5	Trinidad	42·4
112979	F	Cayenne, French Guiana	Flipper tags	Trinidad	55·6	Fr. Guiana	37·8
112954	F	Fishing Pond, Trinidad	Flipper tags	Fr. Guiana	55·2	Trinidad	43·0
25557	M	Grenada/St. Vincent	PTT track	Fr. Guiana	54·3	Trinidad	45·5
77412	F	Matura, Trinidad	Flipper tags	Fr. Guiana	54·2	Trinidad	42·9
112969	F	Cayenne, French Guiana	Flipper tags	Trinidad	52·8	Fr. Guiana	47·2
112972	F	Yalimapo, Amana Nature Reserve, French Guiana	Flipper tags	Trinidad	52·1	Fr. Guiana	47·9
37402	F	Grande Riviere, Trinidad	Flipper tags	Fr. Guiana	51·7	Trinidad	48·0
112964	F	Chiriqui, Panama	Flipper tags	Costa Rica	45·3	Fr. Guiana	27·5
Incorrect							
112946	F	Fishing Pond, Trinidad	Flipper tags	Costa Rica	98·7	Trinidad	1·2
77408	F	Grande Riviere, Trinidad	Flipper tags	St. Croix	94·2	Costa Rica	5·8
112941	F	Sandy Point, St. Croix	Flipper tags	Costa Rica	90·5	St. Croix	9·5
73671	F	Parismina Beach, Costa Rica	Flipper tags	Trinidad	90·3	Costa Rica	5·3
78526	F	Juno Beach, Florida	Flipper tags	St. Croix	83·6	Costa Rica	13·0
25558	F	La Playona, Colombia	Flipper tags	Costa Rica	83·1	St. Croix	11·2
77397	F	Gandoca, Costa Rica	Flipper tags	Trinidad	74·5	Fr. Guiana	19·0
77399	F	La Playona, Colombia	Flipper tags	Costa Rica	73·3	St. Croix	15·6
73653	F	Juno Beach, Florida	Flipper tags	Trinidad	67·2	St. Croix	31·1
51997	F	Carolina, Puerto Rico	Flipper tags	Costa Rica	63·1	St. Croix	17·5
91164	M	Grenada/Leeward Islands	PTT track	Costa Rica	57·1	Trinidad	34·8
77411	F	Gandoca, Costa Rica	Flipper tags	Trinidad	53·3	Fr. Guiana	41·3
25561	F	Chiriqui, Panama	Flipper tags	Trinidad	51·9	Costa Rica	28·8
112973	F	Paria Bay, Trinidad	Flipper tags	Costa Rica	49·6	Trinidad	45·7
44099	F	Babunsanti, Suriname; Point Isere, French Guiana	Flipper tags	Trinidad	49·6	St. Croix	25·9
44105	F	Babunsanti, Suriname; Point Isere, French Guiana	Flipper tags	Trinidad	48·0	Costa Rica	28·8
91182	F	Pacuare Beach, Costa Rica	Flipper tags	Trinidad	46·4	St. Croix	30·0
77400	F	Matura, Trinidad	Flipper tags	St. Croix	38·7	Trinidad	33·1
62590	F	Luri, Guyana; Cipara, Venezuela; Grande Riviere, Trinidad	Flipper tags	Costa Rica	38·2	Fr. Guiana	31·3

Table 6. A summary of the assigments for foraging leatherback turtles using ONCOR, GeneClass2 and Structure, indicating whether assignments were correct, correct within the region, or incorrect

	ONCOR	Geneclass2	Structure
Correct	33	26	27
Regional	31	34	31
Incorrect	19	23	25
% Correct	77·1	72·3	69·9

Atlantic have recently been proposed in eastern Canada. Our goal was to identify the natal origin of turtles foraging in this region. Our robust reference data set of 1417 animals representing nine distinct populations helped us to identify natal beaches for the foraging turtles because each key nesting area in the reference data set represents an epicentre of leatherback nesting in various locations throughout the Atlantic and Caribbean. Each of the nine major nesting assemblages that were sampled is surrounded by smaller nesting beaches; turtles on these beaches are likely to be of the same genetic stock as the main nesting assemblage in the area. Dutton *et al.* (in press) point out, for instance, that St. Croix should be considered as representative of a broader northern Caribbean genetic stock. In addition, some areas may contain finer scale regional structuring that has yet to be characterized, such as the Guiana shield in the eastern Caribbean (Dutton *et al.* in press). We found that the majority of leatherbacks captured in Canadian waters were from the Trinidad and French Guiana nesting aggregations. Other nesting populations in the wider Caribbean had turtles assigned (St. Croix, Costa Rica and Florida). Only one turtle was assigned to Florida, despite additional confirmation of this nesting area as a contributor to the Canadian leatherback foraging population based on tag-recapture data ($n = 2$, Table 5), and satellite telemetry (Eckert *et al.* 2006). No

foraging turtles were assigned to Brazil in the southeastern Atlantic, South Africa, or to Gabon or Ghana in West Africa. Satellite tracks of leatherbacks tagged in Canadian waters are consistent with this finding, and demonstrate strong migration and seasonal residency patterns that are largely limited to Western Atlantic waters, north of the equator (e.g. James, Myers & Ottensmeyer 2005). Recent satellite telemetry studies and analysis of leatherback movements have revealed additional patterns of note. For example, Fossette *et al*. (2010a) found that turtles tracked from French Guiana, Suriname, Grenada, Nova Scotia and Ireland displayed three distinct migration strategies. Turtles exhibited travel patterns that took them either on a round-trip (from where they started), to northern waters or to residence areas in equatorial waters. They found that leatherbacks use northern waters, on both sides of the Atlantic, where productive zones are likely characterized by high concentrations of gelatinous prey. Another study in the Southwest Atlantic (Lopez-Mendilaharsu *et al*. 2009) showed that leatherbacks do not cross the equator to the north, but travel up and down along the continental shelf, similar to leatherbacks in some parts of the Northwest Atlantic (Eckert *et al*. 2006). In addition, turtles nesting in West Africa and those nesting in Brazil migrate to foraging areas in the southwestern Atlantic off the coast of Brazil, Uruguay and Argentina (Almeida *et al*. 2011; Witt *et al*. 2011). There is evidence that adult leatherback migration patterns may reflect the hatchling drift hypothesis (see Hays *et al*. 2010; Fossette *et al*. 2010b; Gaspar *et al*. 2012), in that adults may seek out areas for foraging that they experienced as hatchlings when they were not as able to direct their own movements and relied on ocean currents for transport. Fidelity to foraging areas at high latitudes, such as waters off Canada's Atlantic coast, where small size classes of leatherbacks have yet to be recorded (James, Sherrill-Mix & Myers 2007), may be linked to movements accompanied by foraging success in the histories of individual turtles when they first entered temperate waters. With the conspicuous absence of southern Atlantic or African populations among Canadian tag-recapture data, and the varying migration patterns among turtles from these nesting areas, our results showing only northwestern Atlantic turtles in the Canadian foraging grounds are not entirely surprising. It would be beneficial to examine nesting assemblage contributions to other less well studied foraging regions in the North Atlantic (i.e. Gulf of Mexico, mid-Atlantic oceanic regions and Northeast Atlantic, see Eckert *et al*. 2006; Hays *et al*. 2006; Fossette *et al*. 2010a) to gain a better understanding of the full foraging range and corresponding threats for the species.

Taken alone, tagging, satellite telemetry, or genetics data may provide only limited information about migratory connectivity for transboundary species, so combining these methods is highly recommended (Webster *et al*. 2002; Godley *et al*. 2010). Using telemetry and genetic assignment tests, Abbott *et al*. (2006) showed that shy and white-capped albatrosses, although closely related species, faced different threats from fisheries bycatch because of varying distribution patterns while foraging. Thus, it was essential to define nesting population genetic differences to evaluate the threats from distant fisheries to each species and population. In addition, Abbott *et al*. (2006) were able to assess differences in bycatch threats to males versus females and for albatrosses of different life stages. Evaluating threats for other species with broad distributions may be problematic. Tagging turtles on nesting beaches is labour-intensive and the probability of recapture at the foraging grounds is low. Satellite telemetry is expensive, and most studies suffer from a low sample size from which to infer behaviour (Godley *et al*. 2007). Robust genetic analysis depends on having the correct number of informative markers (Topchy, Scribner & Punch 2004), and a large enough sample data set (as well as reference data sets) so that informative assignments may be made (Manel, Gaggiotti & Waples 2005). Our ability to independently ground-truth our genetic results with tagging and telemetry allowed us to validate our findings and illustrates the benefit of combining data from multiple methods to understand migratory connectivity for highly mobile marine species. Seventy-seven per cent of the recaptured turtles in our sample were assigned correctly to their natal origin. This assignment accuracy is comparable to that in other studies that have used complementary methods for assigning natal origins, for example 76%–80% in neotropical birds (Kelly, Ruegg & Smith 2005).

Similar to the Abbott *et al*. (2006) study, using multiple methods allowed us to assign both sexes and different life stages to natal populations. Consistent with earlier research (James, Sherrill-Mix & Myers 2007), there were more than twice as many females captured in Canada as there were males. A broader comparison study of the migration strategies of male versus female leatherbacks would be useful in ruling out sex-biased dispersal that occurs in other marine species (e.g. dolphins; Möller & Beheregaray 2004). Of nine satellite-tagged male turtles we considered, eight had genetic assignments to a nesting stock that matched their satellite tracks to residence areas likely used for mating (James, Eckert & Myers 2005). The genetic assignment of a single male to Costa Rica matched its satellite track and area of residence. Three other males spent time directly adjacent to Trinidad and were assigned there; another was assigned to French Guiana and that assignment matched its residence area. Three other males were assigned to French Guiana, although they spent their time off St. Vincent and the Grenadines, Grenada and/or St. Lucia. These males may have been staging there to intercept females heading for the northern coast of Trinidad or French Guiana, possibly because there was a high density of females moving through the area, or because the males had previously been successful at mating in that location. Although the genetic connectivity among nesting populations on these islands has not been analysed, these populations may reasonably be assumed to be related to the Trinidad or French Guiana

assemblages to the south. Only one male was considered to have an incorrect assignment. This turtle was assigned to Costa Rica even though it was tracked to and seasonally resident in the Grenada area. Both satellite-tracked females were considered correctly assigned to Trinidad; one spent time directly off Trinidad and Venezuela, while the other spent time off Grenada and St. Vincent, but was not observed nesting. We assumed that satellite-tracked turtles were targeting a beach when they spent time during the nesting season in residence there.

Because the satellite-tracked turtles (both males and females) were assigned correctly to those nesting beaches adjacent their areas of temporary residence, this study represents the first time, for any sea turtle species, that a natal beach has been inferred using satellite tracks and confirmed with genetic assignments. This is particularly important for male leatherbacks, as little is known about their level of natal homing and site fidelity. It was previously hypothesized that male leatherback annual migration and fidelity to breeding areas adjacent nesting beaches was indicative of natal homing (James, Eckert & Myers 2005). The present genetic results provide compelling evidence for philopatry among males.

Only 19 turtles for which we established natal origins using other information were incorrectly assigned in ONCOR. Of these, two turtles observed nesting in Colombia were assigned to Costa Rica. Turtles on nesting beaches in Colombia have not been genetically sampled, and, as there is exchange of nesting individuals between Costa Rica, Panama and Colombia (Ordoñez *et al.* 2007), nesting beaches in all three countries are increasingly considered one assemblage (Orbesen *et al.* 2008). Nine other turtles incorrectly assigned by ONCOR had secondary assignments that matched the beach where they had been observed nesting (e.g. Turtle 77400 assigned to St. Croix with 38·7% probability and to Trinidad with 33·1% probability; the turtle was seen nesting in Trinidad) (Table 5).

Although many leatherbacks nesting at low latitudes in the western Atlantic travel north to Canadian waters following nesting (females) or breeding (males) (James, Eckert & Myers 2005; Eckert 2006; James *et al.* 2006), other female turtles from the same nesting beaches have been tracked to other locations throughout the North Atlantic such as the Bay of Biscay, the Flemish Cap (Eckert 2006; Hays *et al.* 2006), and other oceanic regions (Fossette *et al.* 2010a). Because the Trinidad and French Guiana nesting aggregations are the largest in the west Atlantic (Lee Lum 2005; Girondot *et al.* 2007; S. Eckert, unpublished data), and surpassed in size, ocean basin-wide, only by the West African population (Witt *et al.* 2009), we expected to see a disproportionate representation of these populations in the foraging turtles off Canada, compared to the other nesting assemblages in the Caribbean region. Indeed, 81·9% of the turtles captured in Canada were assigned to either the Trinidad or French Guiana assemblage. Dutton *et al.* (in press) were able to identify 7 Management Units (MUs) for leatherbacks in the Atlantic based on mitochondrial (mtDNA) sequence data, but 9 Demographically Independent Populations (DIPs) based on microsatellite data. Previously, microsatellites did not show much structuring for marine turtle populations, possibly due to male-mediated gene flow (e.g. Bowen *et al.* 2005), but the current finding of structure in Atlantic leatherbacks (Dutton *et al.* in press) suggests that male-mediated gene flow may not be as widespread as previously believed. Dutton *et al.* (in press) found that while Trinidad and French Guiana were not differentiated based on mtDNA haplotype frequencies; they detected a low but highly significantly level of differentiation with their microsatellite data and showed that the mtDNA marker had insufficient power to detect the low levels of differentiation that characterize fine-scale population structure. It is therefore possible that although we considered French Guiana and Trinidad separate DIPs for this study because we used microsatellites for the assignments, there may be ongoing overlap and exchange of females (and males) that provide some degree of connectivity within this region. Indeed, when the assignments were run using only eight DIPs (French Guiana and Trinidad combined), the recaptured turtles assigned with very high probabilities to the combined assemblage of Trinidad/French Guiana (data not shown, but available). This potential overlap in nesting sites is reflected in many of the recaptured turtles (Table 5) having assignment probabilities split between Trinidad and French Guiana (e.g. Turtle 91165 assigned primarily to Trinidad at 57·1% and secondarily to French Guiana at 41·4%) and brings the question of scale into the task of defining the appropriate population unit for management or threat assessments. Similarly, Paetkau, Amstrup & Born (1999) found that although genetic differences were minimal between some populations of polar bears, telemetry showed that there was overlap in the populations and this subsequently affected the designation of management units for the species.

The number of nesting females in Trinidad ranges from 7000 to 12 000 per year (S. Eckert unpublished data), French Guiana has an estimated 1342–3000 nesting females per year (Girondot *et al.* 2007), and Suriname has between 1545 and 5500 nesting females per year (Hilterman & Goverse 2007). Therefore, collectively, these nesting assemblages number between 9887 and 20 500 females each year, while other rookeries represented in this study have far fewer females and had significantly lower representation among the Canadian-captured turtles. Costa Rica, which had the next highest number of turtles assigned, is the second largest rookery in the Caribbean after Trinidad, and supports 5759–12 893 nests per year (~ 2000 nesting females; Troëng, Chacón & Dick 2004), while St. Croix and Florida each have ~500–1000 nesting turtles annually (Dutton *et al.* 2005; Stewart *et al.* 2011). However, these populations show strong positive trends in growth (Dutton *et al.* 2005; Stewart *et al.* 2011), and we might expect to see the proportions of these rook-

eries increasing in coming years in the samples from Canada. The genetic assignments in this study, and the composition of the Atlantic Canadian leatherback foraging population, currently reflect relative population sizes for rookeries throughout the wider Caribbean, thus identifying the migratory connectivity between Canadian high-latitude foraging areas and low-latitude breeding areas for this species.

During late spring through fall, mature female leatherbacks from nesting areas throughout the western Atlantic converge to forage on scyphomeduase off Canada's coast. Here they join mature male and sub-adult leatherbacks, which undertake annual return migrations to Canadian waters (James, Ottensmeyer & Myers 2005; James, Eckert & Myers 2005). The fidelity of leatherbacks to high-latitude foraging areas encompassing Canadian waters, their extended seasonal residency there, and the demonstrated high energetic consumption by turtles feeding in these areas (Heaslip *et al*. 2012), highlight the great importance of this part of the world to this species. Recognizing the endangered status of leatherbacks in the Atlantic, and the vast distances these animals travel between breeding and feeding areas, there is a pressing need for bilateral and multilateral cooperation and communication to achieve species conservation and recovery objectives. International collaboration, facilitated by the Internet, along with dedicated research teams, has resulted in the sharing of tag-recapture data collected from animals encountered in Canadian waters and on nesting beaches.

Currently, many of the key international agreements focused on sea turtle conservation have been principally developed for, and supported by countries that host nesting populations. However, there is increasing emphasis on the role that countries with jurisdiction over key foraging habitat can, and should play in at-sea monitoring and promotion of species recovery. For example, the recent heightened interest in evaluating and mitigating sea turtle bycatch on the part of the International Commission for the Conservation of Atlantic Tunas (ICCAT) has prompted several countries, including Canada, to expand sea turtle research initiatives. An international recovery plan might be developed by members of parties that are signatories to the Inter-American Convention for the Protection and Conservation of Sea Turtles (IAC), although many countries with turtle nesting beaches (and/or foraging grounds) have still not signed on to the Convention.

This study highlights the importance of using fishery-independent data to ground-truth and assess threats to sea turtles, as well as the importance of continuing monitoring and tagging programmes throughout nesting beaches in the Caribbean, and in key foraging areas. By providing concrete, proportional linkages between specific western Atlantic leatherback rookeries and Canada's foraging grounds, evaluation of the implications of anthropogenic mortality in this critical high-latitude foraging area may now be more readily estimated, compelling the rapid assessment and mitigation of threats to leatherbacks in Canadian waters.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. GeneClass2 assignment test results for 288 turtles foraging off Nova Scotia Canada. Most of the turtles were assigned to Trinidad, but all other rookeries were well-represented.

Table S2. Structure assignment test results for 288 turtles foraging off Nova Scotia Canada. French Guiana had the greatest number of turtles assigned.